AFCEC-CX-TY-TR-2016-0015

TOXIC AEROSOLS AND PATHOGENIC BIOAEROSOLS

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Contract No. FA4819-11-C-0003

April 2016

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AFCEC-201621, 17 August 2016

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OMB No. 0704-0188

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1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
06-04-2016	Technical Report	1/3/2012 - 3/31/2014

4. TITLE AND SUBTITLE

Toxic aerosols and pathogenic bioaerosols

5a. CONTRACT NUMBER

FA4819-11-C-0003

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Charles Balzli (UTC), April Lumley (UTC), Brian Duval (UTC), Bob Nichols (UTC), Rashelle McDonald (UTC),
Brian Heimbuch (ARA), Delbert Harnish (ARA), Michael McDonald (ARA)

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Universal Technology Corporation (UTC), 1270 N. Fairfield Road, Dayton, OH 45432
Applied Research Associates (ARA), 430 W 5th St, Panama City, FL 32401

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Air Force Civil Engineer Center
Readiness Directorate
Requirements and Acquisition Division
139 Barnes Drive, Suite 2
Tyndall Air Force Base, FL 32403-5323

10. SPONSOR/MONITOR'S ACRONYM(S)

AFCEC/CXA

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

AFCEC-CX-TY-TR-2016-0015

12. DISTRIBUTION/AVAILABILITY STATEMENT

A: Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

Document contains color images. Reference Public Affairs Case #201621, 17 August 2016.

14. ABSTRACT

This report summarizes the findings of four investigations of interactions of viable microbes with filter media. The first, challenging N95 filtering facepiece respirators (FFRs) separately with 800- and 100-nm latex and viral aerosols, showed experimentally that the media do not discriminate between inert and biological particles of either size. The second designed and built an artificial breathing system capable of operation at rates from 10 to 400 L/min, to complement an articulated headform separately generated. The third generated and delivered an aerosol of MS2 coli phage through an iodinated filter medium into 3-L plastic volumes prepared from food storage bags, incubated the aerosols for 1 to 10 minutes at room temperature and low, medium or high humidity, collected the aerosol in impingers containing thiosulfate to terminate the activity of iodine, plated the the contents of the impinger and measured viable MS2 counts on the plates. This showed that the half-life for viable MS2 airborne after exposure to iodine is ~1 min---too short to be useful for FFRs, but of possible value in HVAC contexts. In the final study, volunteer cleaning staff members at a local hospital wore N95 FFRs while cleaning vacated patient rooms---the first study using humans as the collectors in an environmental study. Collection and plating of captured bacteria showed that the air clearance of fine particles is efficient, that a measurable viable population remains in the form of particles ~10 micrometers, that the majority of the species assayed showed antibiotic resistance to oxacillin, and that ~20% showed resistance to vancomycin as well.

15. SUBJECT TERMS

influenza, bioaerosol, filtration, particle size distribution, nebulizer, respirator

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Michael V. Henley
U	U	U	SAR	32	19b. TELEPHONE NUMBER *(Include area code)*

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1.0 EXECUTIVE SUMMARY

This report describes technical effort provided by Universal Technology Corporation (UTC) under prime contract FA4819-11-C-0003 to the Air Force Research Laboratory (AFRL/RXQL) and under later transition to the Air Force Civil Engineer Center (AFCEC/CXA), and includes a subcontract effort to Applied Research Associates (ARA) from 3 January 2012–31 March 2014. The technical program was composed of discrete projects in support of varied U.S. DoD or U.S. Department of Health and Human Services (DHHS) requirements and each is described separately in this document. Technical work in toxic and pathogenic biological aerosols encompassed research and development in the following four primary areas;

1. *Filtration Study Using Viable H1N1 Aerosols*

Guidance on infection control for the care of patients with confirmed or suspected H1N1 influenza infections recommends the use of a fit-tested disposable N95 respirator. Studies were conducted to validate the filtration efficiency of Filtering Facepiece Respirators (FFR). All FFR demonstrated filtration efficiencies exceeded the N95 benchmark ratings. Tests were conducted with inert polystyrene latex (PSL) beads as a model system, and with viable H1N1 influenza aerosols.

2. *Total Inward Leakage Study Using Viable H1N1 Aerosols*

A study was conducted with viable H1N1 aerosols and filtering respirators to determine potential leak points of FFR. A custom designed aerosol exposure system was developed and fabricated, composed of an artificial breathing machine, a static headform, and a containment chamber. The system was validated by sampling from specified locations around the chamber. Testing was not completed on this system, however, due to delays in development and testing and budget constraints.

3. *MS2 Challenge of Reactive Media*

The effect of relative humidity (RH) on transmission of MS2 bacteriophage through a Triosyn Super High-efficiency (TSH) particulate air (HEPA) filter was completed at ~50% and ~30% RH and confirmed that viability of phage penetrating the TSH filter increased with increasing humidity levels. The percent viability downstream of the filter varied depending upon specific test conditions.

4. *FFR Hospital Wear Assessment*

In parallel studies, the number of bacterial isolates was obtained from FFRs worn during cleaning tasks in areas that were temporarily free of patients and tested for antibiotic resistance. Results showed both oxacillin- and vancomycin-resistant strains were readily isolated from hospital environments.

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2.0 FILTRATION STUDY USING VIABLE H1N1 AEROSOLS

2.1 Technical Introduction

The 2009 H1N1 influenza pandemic spotlighted the urgent need to establish effective infection control methods to confine the pandemic. H1N1 is an airborne, communicable disease that is readily spread from person to person. The device recommended for protecting individuals from inhaling pathogenic aerosols is the National Institute for Occupational Safety and Health (NIOSH)-approved N95 FFR. Occupational Safety and Health Administration (OSHA) and the Centers for Disease Control and Prevention (CDC) recommend healthcare workers wear a properly fitted, NIOSH-approved FFR when attending to patients with influenza symptoms [1]. The purpose of this study was to determine if N95 FFRs remove viable H1N1 influenza with the same efficiency as inert particles of the same size. Evaluation of the 0.8-μm particle size was performed previously at 85-liter-per-minute (LPM) flow rates, and initial studies were conducted at 170 LPM [2]. The study reported herein describes completion of the 170-LPM data set and evaluation of 0.1-μm particles at 85 LPM.

2.2 Methods

2.2.1 Preparation of H1N1 Virus

Influenza A/PR/8/34 VR-1469 (ATCC VR-95) was propagated in embryonic chicken eggs using standard protocols [3]. Virus titers were determined using a median tissue culture infective dose ($TCID_{50}$) assay in Madin–Darby canine kidney cells (MDCK; ATCC CCL-34) with cell culture techniques approved by the World Health Organization (WHO) [3].

2.2.2 Aerosol Test Conditions

Three aerosol test conditions were originally proposed to challenge the FFRs with influenza virus**.** All were based on standard methods for challenging air purification devices with viable aerosols. Condition #1 has already been performed using a flow rate of 85 LPM (Table 1).

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Table 1. Conditions #1 for Challenging FFRs with H1N1

	Condition #1	Condition #2	Condition #3
Viral strain	Influenza A/PR/8/34 VR-1469 (ATCC VR-95)		
Flow rate, LPM	85	170	85
Temperature, Humidity	~23 °C, 35–50% RH		
Particle size, μm CMD	0.8		0.1
Aerosolization media	Artificial saliva		0.5% Mucin
Aerosolization device	LSAT		
Atomizer	Collison nebulizer		
Viable collector	AGI-30		
Collection buffer	Serum-free EMEM		
Viable assay	$TCID_{50}$ assay in MDCK cells		
Replicate measurements	3 FFRs, 3 upstream and downstream measurements for each FFR		
Duration of viable sampling	5 min per impinger		
Quality control	Leak check FFRs, background check on LSAT, stable aerosol check, pressure drop		

Note: *CMD (count median diameter), *AGI (all glass impinger), serum-free *Eagle's minimum essential medium (EMEM) may contain neutralizers for antimicrobial FFR
Condition #2 used a flow rate of 170 LPM to provide a more rigorous challenge. Condition 3# used the standard 85-LPM flow rate delivering a challenge of 100-nm particles.

2.2.3 LSAT Setup

A Laboratory Scale Aerosol Tunnel (LSAT) was previously designed to challenge air purification systems with viable microbial aerosols and was ideally suited for this study [4] (Figure 1). A complete description, operation instructions, validation report, and accompanying test protocols have been previously described [5].

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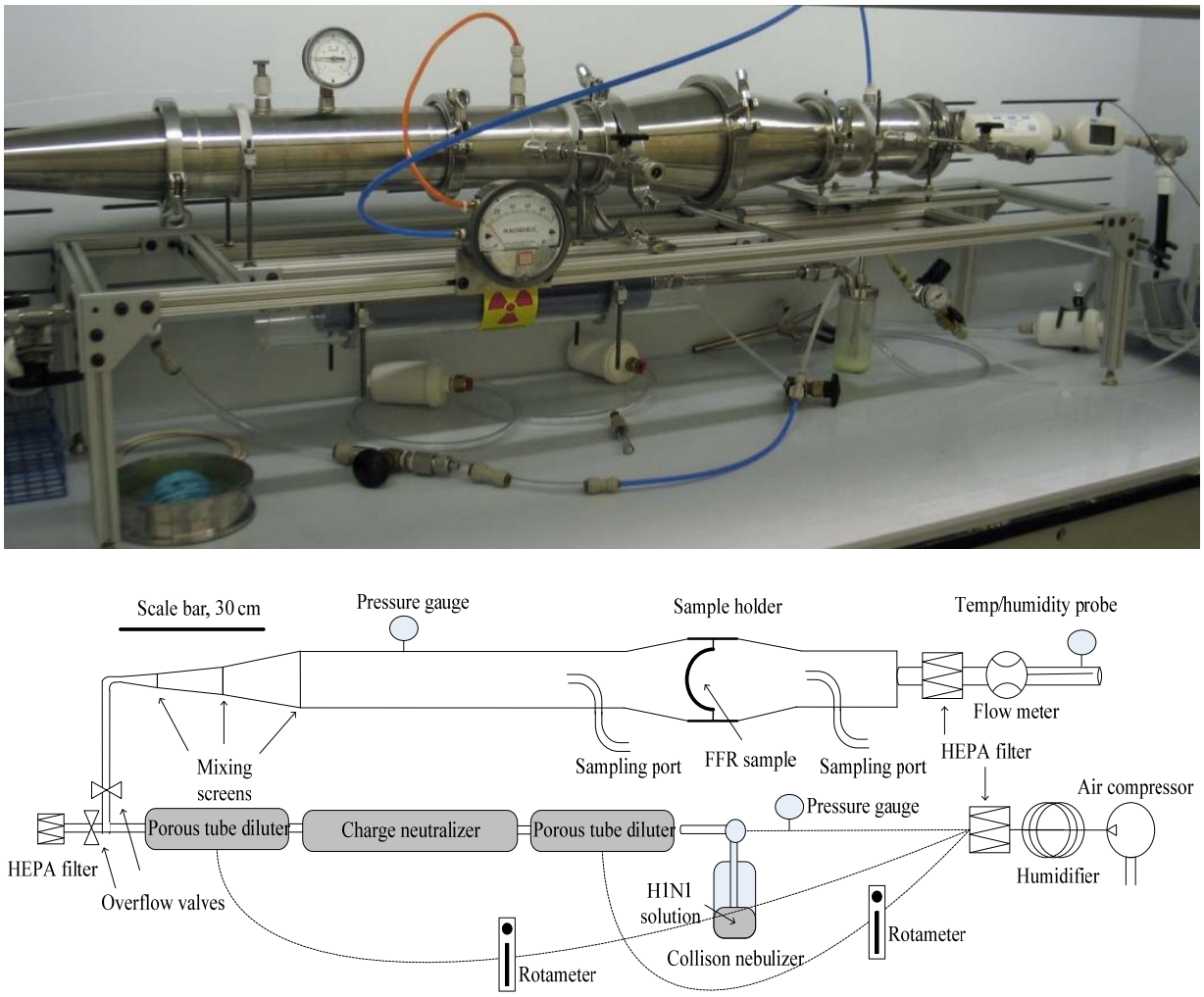

Figure 1. LSAT System

Briefly, the LSAT is composed of 10-cm diameter stainless steel sanitary fittings; a 15-cm diameter filter holder is used to accommodate the FFR. The biological aerosol is generated using a six-jet Collison nebulizer (BGI Inc., Waltham, MA). Dilution air is conditioned by passage through a humidifier, and added through two porous-tube diluters (Mott Corporation, Farmington, CT), one located upstream and the other downstream of the charge neutralizer. The Kr-85 charge neutralizer (TSI Inc., Shoreview, MN) is required to counteract charges created on particles during aerosolization. To divert aerosol away from the test specimen, overflow valves are located upstream of the expansion chamber. The expansion duct contains three mixing screens that create turbulent flow and allow the aerosol to mix prior to being exposed to the test specimen. Isokinetic sampling ports located upstream and downstream of the sample allow for viable sampling of microbial agents from the airstream and can also be used with traditional particle counters.

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A critical mechanical element during operation of the LSAT is to ensure the upstream and downstream sampling ports collect the same volume of particles. To validate the performance of the sampling ports for Condition #1, 30 mL of artificial saliva buffer [6] (0.42 g $NaHCO_3$, 0.04 g $MgCl_2$•7 H_2O, 0.13 g $CaCl_2$•H_2O, 7.70 mL 0.2 M KH_2PO_4, 12.3 mL 0.2 M K_2HPO_4, 0.11 g NH_4Cl, 0.19 g KSCN, 0.12 g $(NH_2)_2CO$, 0.88 g NaCl, 1.04 g KCl, and 3.00 g mucin (Sigma–Aldrich, St. Louis, MO, M1778) in 1 L deionized water, pH 7) was placed in a six-jet Collison nebulizer and attached to the LSAT. Compressed air (20 psi) was added to the Collison nebulizer to start the aerosol flow. Dilution air was added to both porous tube diluters to maintain the total flow at 85 LPM. For Condition #2, the total flow was adjusted to 170 LPM. For Condition #3, 0.5% mucin was used rather than the artificial saliva mixture.

The LSAT was run for 10 min (30 min for Condition #2), then samples were taken alternately from the upstream and downstream ports using an Aerodynamic Particle Sizer (APS; 3321, TSI Inc., Shoreview, MN) and a Scanning Mobility Particle Sizer (SMPS; TSI Inc., Shoreview, MN). Three upstream and three downstream measurements were collected. The port correlation was repeated three additional times using an aerosol of 0.8- and 0.1-µm PSL beads (Thermo Scientific, Waltham, MA). The particle size distribution (PSDs) produced by 0.8-µm PSL beads and artificial saliva buffer are compared in Figure 2; The PSDs produced by 0.1-µm PSL beads and 0.5% mucin suspension are compared in Figure 3.

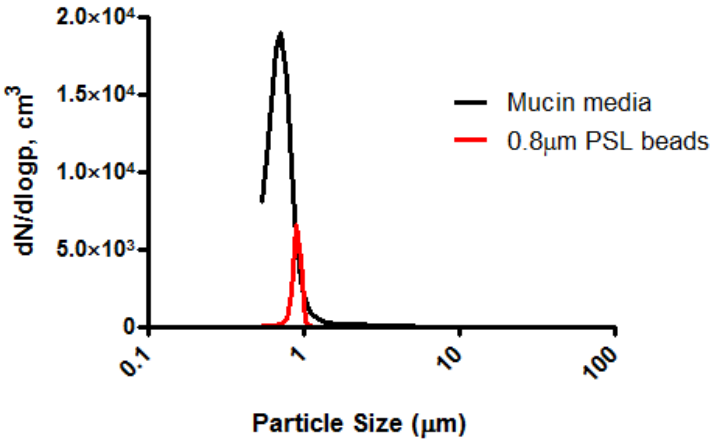

Figure 2. Comparison of Artificial Saliva PSD to 0.8-µm PSL Bead PSD

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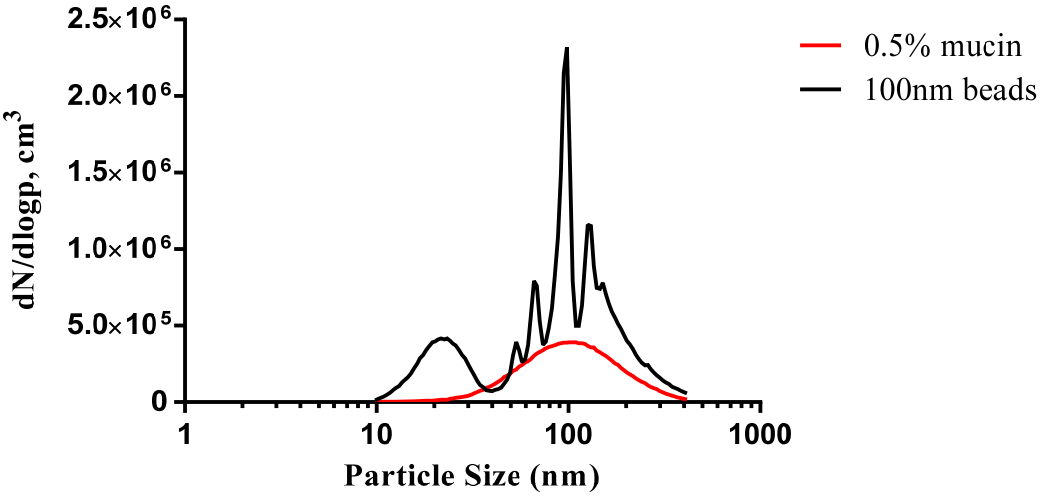

Figure 3. Comparison of 0.5% Mucin PSD to 0.1-µm PSL Bead PSD

2.2.4 Preparation of Filtering Facepiece Respirators

Three surgical FFRs common to hospital settings were selected for the study: 3M™ 1860S, 3M™ 1870, and Kimberly Clark FFR. Also selected were two FFRs that contain an antimicrobial coating: Triosyn T5000 (Safe Life®) and GlaxoSmithKline (GSK) Actiprotect. Three replicate samples of each FFR were glue-sealed into 15-cm sample holders. The filters were leak checked by challenging each filter with an aerosol composed of either 0.8-µm (Condition #2) or 0.1-µm (Condition #3) PSL beads as previously described.

2.2.5 H1N1 Filtration Studies

Prior to each test, the LSAT was flushed with HEPA-purified air for 30 min, after which a minimum of three APS measurements were taken on the upstream and downstream port. A leak-checked FFR was loaded into the LSAT using sanitary compression seal fittings. The six-jet Collison nebulizer, containing 1 mL of H1N1 influenza virus (~8 $\log_{10}TCID_{50}$ per mL) suspension diluted into 30 mL of artificial saliva buffer or 0.5% mucin, was attached to the LSAT. The LSAT was configured to direct the aerosol to the overflow and not to the FFR. Compressed air (20 psi) was applied to the nebulizer and dilution air was added to both porous tube diluters, so the total flow was 85 LPM. The system operated for 10 min to bring the nebulizer to steady state, whereupon the LSAT overflow valves were reconfigured to direct the viral aerosol to the FFR sample for 5 min. Viable sampling of the aerosol via upstream and downstream ports was initiated by connecting an all-glass impinger (AGI-30; Ace Glass, Vineland, NJ), containing 20 mL of serum-free Eagle's minimum essential medium (EMEM; Hyclone Laboratories Inc, Logan, UT) supplemented with 1% penicillin/streptomycin (Sigma–Aldrich) and 1% L-glutamine to the LSAT. The AGI-30s were directly attached to the isokinetic sampling ports on the LSAT to minimize particle loss. Sampling was started by opening the valve on the isokinetic sampling port, while simultaneously applying vacuum to the AGI-30, which sampled at ~12.5 LPM. After 5 min, the isokinetic sampling port was closed, and the vacuum was deactivated. The AGI-30 samples were transferred to 50-mL centrifuge tubes and placed on ice until viable plating was performed. A total of six samples (three upstream, three downstream) were collected for each FFR.

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2.2.6 Viable Plating of H1N1 Influenza Virus

The viability of H1N1 in sf-EMEM buffer from the AGI-30s was evaluated using a $TCID_{50}$ assay in MDCK cells as described previously. The upstream samples were serially diluted 1/10 to 10^{-6}. The 10^{-2} through 10^{-6} dilutions were plated in quadruplicate into 24-well tissue culture plates containing a confluent lawn of MDCK cells. The downstream samples for the N95 FFR were serially diluted to 10^{-4}, and plated in quadruplicate. The plates were incubated for 4 days at 5% CO_2 /37 ºC prior to reading cytopathic effects and crystal violet—glutaraldehyde staining.

2.2.7 Data Analysis

2.2.7.1 Sampling Port Correlation Factor (CF)

Port correlation with 0.8-µm bead studies (Condition #2) used the APS particle size bins ranging from 0.723–0.925 µm, while the 0.1-µm bead studies (Condition #3) used the SMPS particle bins ranging in size from 0.0947-0.1094 µm. Each port was sampled in triplicate, alternating between upstream and downstream. The counts for these bin ranges were summed to yield a representative particle concentration that included the CMD of the PSD. The port correlation was determined by calculating the ratio of the average downstream counts to the average upstream counts and was required to be >96%.

2.2.7.2 Filtration Efficiency

Upstream and downstream measurements for the 0.8-µm bead study were collected using the 0.723–0.925 µm bins on the APS as described previously [7], and the concentration of the 0.1-µm bead aerosol was determined using the 0.0947—0.1094 µm size bins on the SMPS. Viable virus collected in the upstream and downstream AGI-30s (viable virus per mL of extract) were determined using the Spearman–Kärber formula [8]. Equation 1 was used to determine the total amount of virus recovered from each sample (L_s), in which L is the amount of viable H1N1 expressed in units of $\log_{10}TCID_{50}$/mL, and V is the sample volume (20-mL impinger volume). The viable filtration efficiency (VFE) of the FFRs was determined using Equation 2, in which DLs are downstream $\log_{10}TCID_{50}$ values, ULs are upstream $\log_{10}TCID_{50}$ values, CF is the correlation factor, and n is the number of determinations. The inert particle filtration efficiency (PFE) of the samples were determined using Equation 3, in which U is the upstream particle concentration, and D is the downstream particle concentration. Prism 6 software (GraphPad Inc., La Jolla, CA) was used to determine 95% confidence intervals for the filtration efficiency.

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$$L_S = 10^{[L+\log(V)]} \quad (1)$$

$$VFE = \left(\sum_{i=1}^{n}[1 - (DL_i/UL_i)/CF]\,(100\%)\right)/n \quad (2)$$

$$PFE = \left(\sum_{i=1}^{n}[1 - (D_i/U_i)/CF](100\%)\right)/n \quad (3)$$

$$\text{Virus concentration/sample*} = L_S = 10^{[L + \log(V)]} \quad (4)$$

Where : L = Viable H1N1 expressed in units of $\log_{10}TCID_{50}$/mL
V = sample volume.
* If no viable viruses are present ($L = -\infty$) then Ls will be 0

2.2.7.3 Statistical Analysis of Penetration Data
A two-tailed, paired *t*-test was used to compare the non-viable (beads) and viable (H1N1 influenza) filtration data for the three replicates of each N95 FFR. The mean PFE and VFE values for each FFR were loaded into Prism 6 software to perform the *t*-test at 95% confidence intervals. The mean PFE and VFE values for the correlating conditions (Conditions 1 and 2, Conditions 1 and 3) were compared using a two-tailed, unpaired *t*-test.

2.3 Results

The average upstream challenge for all FFR replicates ranged from 0.88 – 1.8 x 10^3 $TCID_{50}$ per liter of air. The two remaining GSK Actiprotect replicates were tested using Condition 2 parameters (Table 2). Under these parameters, PFE for all FFR models ranged from 98.37% to 99.994%, and the VFE ranged from 96.29% to 99.995%. The only significant difference between PFE and VFE was demonstrated by the Kimberly-Clark model (Table 2).

Table 2. Average Removal Efficiencies of 0.8-µm Particles at 170 LPM

FFR Model	Inert	Viable	*P*
3M 1860S	99.37% ± 0.39%	98.56% ± 0.87%	0.13
3M 1870	99.96% ± 0.03%	99.59% ± 0.27%	0.14
Kimberly–Clark	98.37% ± 0.32%	96.29% ± 0.56%	0.02
SafeLife T5000	99.994% ± 0.009%	99.995% ± 0.002%[a]	0.90
GSK Actiprotect	99.23% ± 0.15%	96.29% ± 2.49%	0.09

[a]Lower limit; the data of all three FFR replicates were below the detection limit.

A comparison of Condition 1 and Condition 2 data was conducted. The Kimberly–Clark model demonstrated significantly different filtration efficiencies for both inert and viable aerosol challenges ($P = 0.003$ and 0.002, respectively).The GSK Actiprotect model was found to demonstrate a significantly different inert particle filtration efficiency ($P < 0.001$).

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All five FFR models were tested under Condition 3 parameters (Table 3). No significant difference between viable and inert particle filtration was observed for any FFR model. A comparison of Condition 1 and Condition 3 data was conducted. The Kimberly–Clark model demonstrated the only significantly different PFE; no significant differences were found for VFE between these two conditions (Table 3).

Table 3. Average Removal Efficiencies of 0.1-µm Particles at 85 LPM

FFR Model	Inert	Viable	*P*
3M 1860	99.42 ± 0.41%	99.26 ± 0.61%	0.49
3M 1870	99.88 ± 0.06%	99.52 ± 0.48%	0.34
Kimberly–Clark	99.17 ± 0.27%	99.43 ± 0.53%	0.49
SafeLife T5000	99.995 ± 0.003%	99.997 ± 0.003%	0.63
GSK Actiprotect	99.64 ± 0.19%	99.84 ± 0.07%	0.26

2.4 Discussion

The data in this study clearly demonstrate that all five models of N95 FFRs are effective at removing viable H1N1 particles from the air stream, exceeding their rating in all cases. The effect of flow rate on N95 FFR performance was assessed by incorporating two flow conditions into the experimental design. According to 42 CFR 84 subpart K, section 84.181, the 85-LPM flow rate is the condition specified by NIOSH for evaluating the performance of FFRs. This flow rate was selected to represent a worker's inhalation at a high work rate. However, peak inhalation flow during breathing may be greater than 85 LPM for brief periods of time and exacerbated further as work intensity is increased. For these reasons, we also tested at 170 LPM to provide an extreme challenge to the filter. Despite three significantly different filtration efficiencies between the two flow rates, critical inspection of the data shows that the actual difference in filtration performance between the 85- and 170-LPM conditions for the particle size studied is negligible (1%~2%). Although statistically significant, these differences are merely an indicator of low variability in the data sets and not a physically meaningful distinction.

The filtration efficiency of aerosols is highly dependent on particle size, as demonstrated by past studies and predicted by filtration theory [9]. The upper range of most penetrating particle size values reported for electret N95 FFRs is ~0.1 µm, the particle size used for Condition 3 [10]. For this particle size, all five FFR models demonstrated PFE and VFE values >95%, the NIOSH benchmark for N95 certification. When comparing the 0.1- and 0.8-µm data, the Kimberly–Clark model showed a statistically, but not practically, significant difference in PFE [2]. No significant differences were observed for VFE. This study demonstrates that the N95 FFR models tested remove particles from the airstream regardless of viability. Particles that contain H1N1 influenza and inert particles of the same size are filtered with equal efficiency. Thus, testing for PFE in lieu of VFE, or biological filtration efficiency (BFE) is sufficient for determining FFR efficacy [11].

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2.5 Conclusions

The primary focus of this study was to determine if N95 FFRs removed H1N1 influenza particles with the same efficiency as inert particles of the same size. At present, all five FFR types have demonstrated filtration performances that meet their benchmark N95 rating for both inert PSL bead and H1N1 influenza aerosols. The data analysis indicates that filtration efficiency is not dependent on a particle's nature of origin, but more on the PSD of the aerosol challenge.

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3.0 TOTAL INWARD LEAKAGE STUDY

3.1 Technical Introduction

Fit testing is a requisite process for ensuring the proper level of protection from an FFR is obtained. Inward leakage of contaminants into an FFR has been described as a combination of leakage through 1) the face seal, 2) the filter element, 3) the exhalation valves (for FFRs so equipped), and 4) other sites (e.g., areas where head straps are connected to the FFR by staples, stitching, and so on) [12]. However, FFR fit has been shown to be the principal source of inward leakage [1]. A test system using a static headform manikin was designed and validated to simulate human exposure to aerosolized pathogens and evaluate the effect of FFR fit on potential exposure to airborne pathogens.

3.2 Methods

3.2.1 Aerosol Test System

A breathing machine schematic was developed for testing N95 FFR fit (Figure 4). This breathing machine was used with a static headform manikin capable of inhaling and exhaling at rates representative of the full range of human breathing, including normal breathing with a mean minute volume of 10 LPM at a respiration rate of 13 breaths/min, and heavy breathing at 150 LPM and a mean respiration rate of 75 breaths/min. The maximum instantaneous flow will be at least 400 LPM.

Figure 4. Breathing Machine

The flow schematic (Figure 5) illustrates the use of a combined pressure/vacuum pump to provide the breathing power. Accumulator tanks store vacuum air on the left tank and pressurized air on the right tank. These tanks minimize pressure fluctuation during the breathing cycle and reduce the required size of the compressor. A three-way proportional control valve (FC-1) controls the pressure in the trachea by connecting to the vacuum or the pressure tank and

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adjusting the valve opening to provide the instantaneous pressure required for the desired flow rate. The flow rate in the trachea is measured using a hot-wire anemometer (TSI, Shoreview, MN) located at the trachea connection. The desired pressure will be input to the valve command connection from an instrumentation-and-control module (NI USB 6210, National Instruments, Austin, TX). The flow control valve is a pilot-actuated valve and requires a separate pressure source for actuation, which is provided through the regulator REG-1.

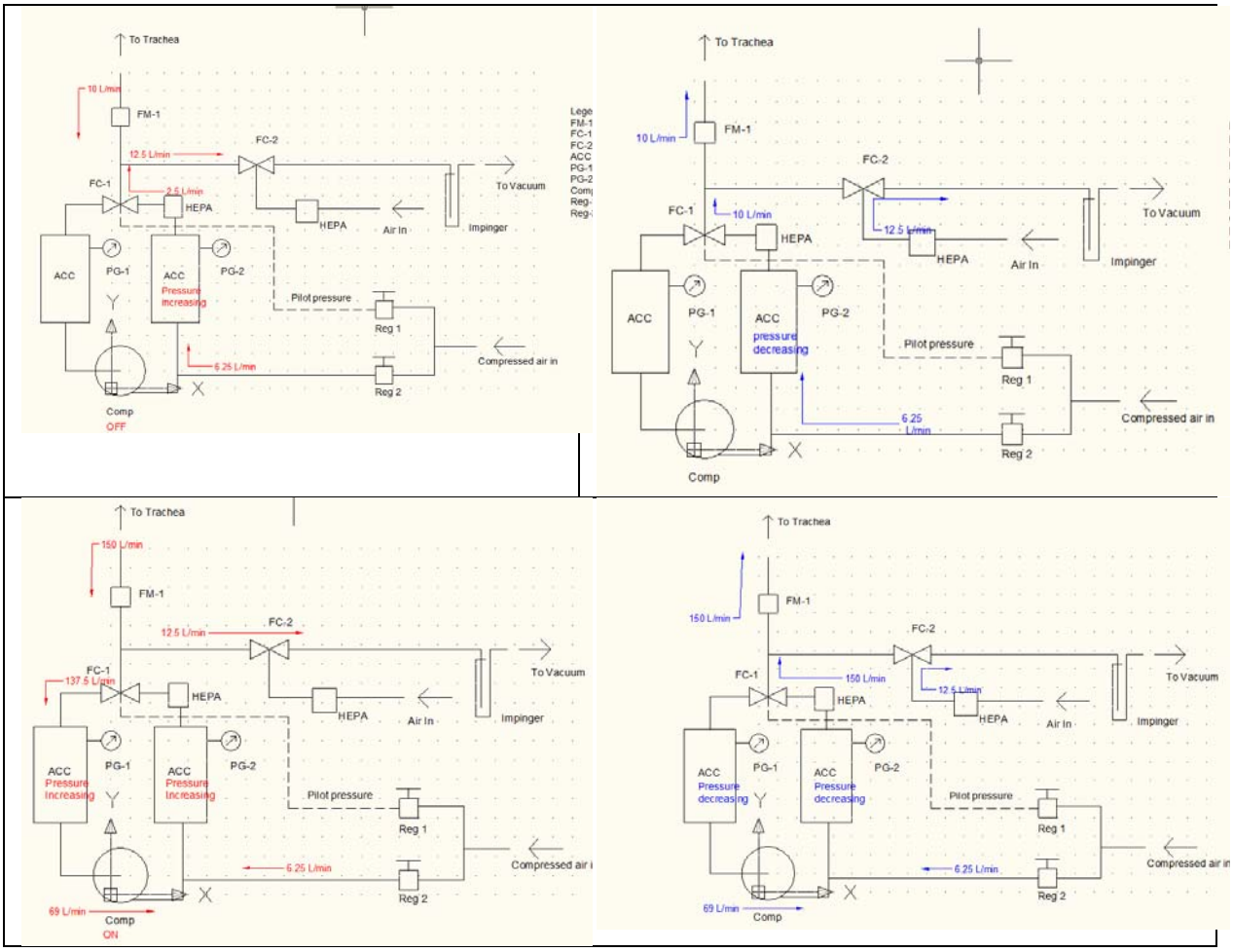

Figure 5. Flow Schematic for Breathing Machine Used to Evaluate N95 FFR Fit Test

In addition, an impinger is included to collect and measure particles (and, when the opportunity arises, gases or vapors) that pass through or around the respirator. The impinger samples only during the inhalation portion of the breathing cycle. During the exhalation portion of the cycle, the impinger draws air from the surrounding area; all effluents from the impinger subsequently pass through a HEPA filter. A two-position, three-way valve (FC-2) isolates the impinger from the breathing machine during the exhalation cycle. The impinger draws air continuously, and as a result, additional air must be introduced into the breathing machine to assure that the respirator is exposed to the same volume of air during the inhale and exhale portions of the breathing cycle. This is accomplished by supplying the appropriate volume of air through REG-2.

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The block diagram in Figure 5 shows the flow direction and magnitude during the inhalation and exhalation portions of the breathing cycle at 10 LPM and 150 LPM. For flows less than 12.5 LPM, the system is powered entirely by the impinger vacuum and compressed air inlet. The vacuum tank and the compressor are not used. At flows greater than 12.5 LPM, the compressor is operational, so the vacuum and pressure tanks as well as the compressor, the pressure inlet, and the impinger vacuum will be coordinated by a LabVIEW™-controlled routine to provide the necessary flows.

3.2.2 Validation protocol

The breathing system was validated for mechanical performance to ensure that it could meet the specified breathing flow-rate requirements. The headform enclosure was challenged with 0.8-µm PSL beads and measurements were taken over a 45-min period at several locations within the enclosure (Figure 6) to evaluate loading consistency over time using an APS. Three independent runs were performed for each sampling point. The sampling points were chosen by taking into consideration the location of the respirator seal and skin interface as well as penetration through the respirator material during breathing. The concentration of beads was measured during both 50- and 80-LPM exposures, which correlate to the flow rates for normal and deep breathing, respectively. Humidity and temperature measurements were also taken the entire duration of the run.

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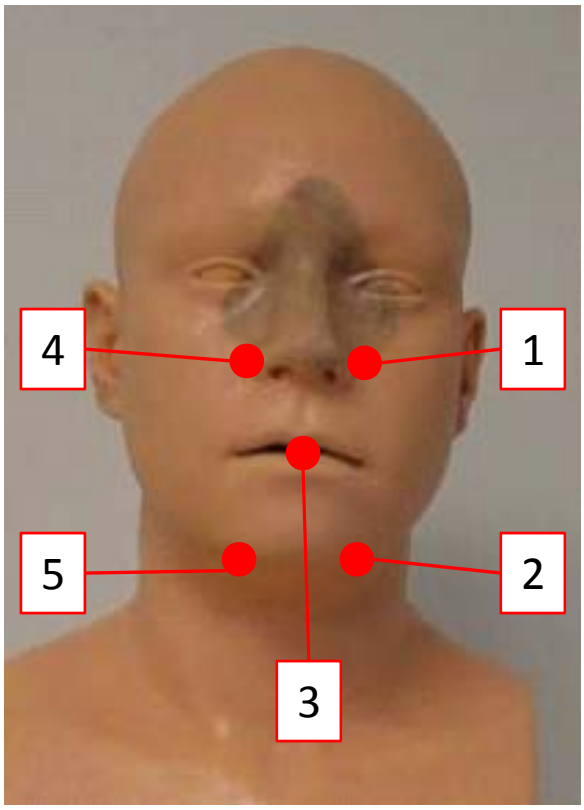

Figure 6. Five Sampling Locations for Validation Testing
Points 1, 2, 4, and 5 were 0.5–1.0 in from the surface of the manikin. Point 3 was 1.5–2.0 in directly in front of the mouth of the manikin.

To evaluate the ability of the system to sample particles upstream of the N95 (in the chamber containing the headform) and downstream of the headform (in the breathing machine) the chamber was filled with 0.8-um PSL beads, and an APS was used to sample over time. Alternate samplings between the upstream and downstream ports were performed to limit sampling bias. Following the inert particulate study, the system was challenged with H1N1 influenza, and AGI-30s were used to sample through the upstream and downstream ports. Protocols for preparing and assaying H1N1 influenza are previously described in this report. Both normal breathing and deep breathing conditions were evaluated.

3.3 Results and Discussion

Initial particle concentration measurements were taken at five minutes and the average concentrations (± standard deviation) for each point were calculated (Figures 7 and 8). The change in concentration of 0.8-µm beads (± standard deviation) over time was measured and is shown in Figures 9 and 10. Small differences in initial concentrations may be attributed to the variation of bead concentration within the Collison nebulizers used for aerosol generation. The more important information for validation purposes is that the concentrations at each point follow the same trend of rate change over time and relative to each other.

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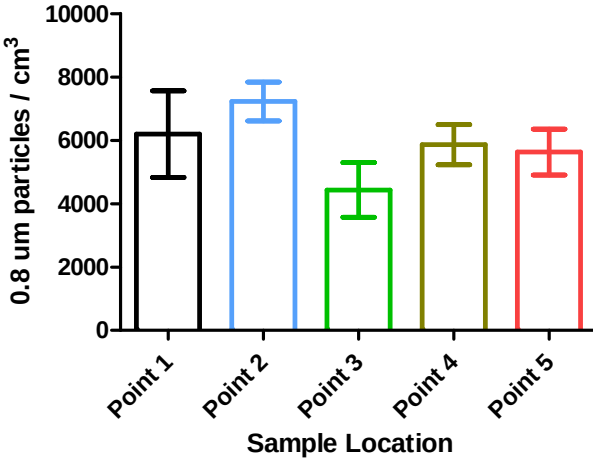

Figure 7. Initial Concentrations of 0.8-µm Beads at Five Different Points During a 50-LPM Challenge (*n* = 3)

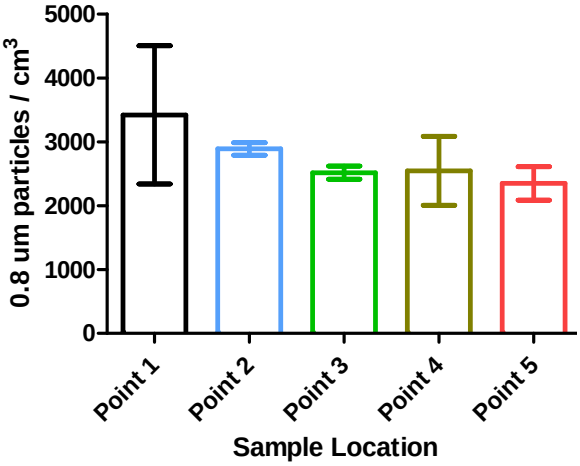

Figure 8. Initial Concentrations of 0.8-µm Beads at Five Different Points During an 80-LPM Challenge (*n* = 3)

The RH and temperature of the 50- and 80-LPM aerosol challenges were measured with and without breathing. The RH of the challenge slightly decreases over time (Figures 11 and 12) likely due to the dryer air used to dilute the aerosol and increase the overall flowrate of the challenge. This is also demonstrated by the overall lower RH of the 80-LPM challenge compared to the 50-LPM challenge because the 80-LPM challenge is diluted with more "dry" air from the compressed air source. There is some variation between individual runs, which is likely due to the day-to-day changes in ambient air of the laboratory.

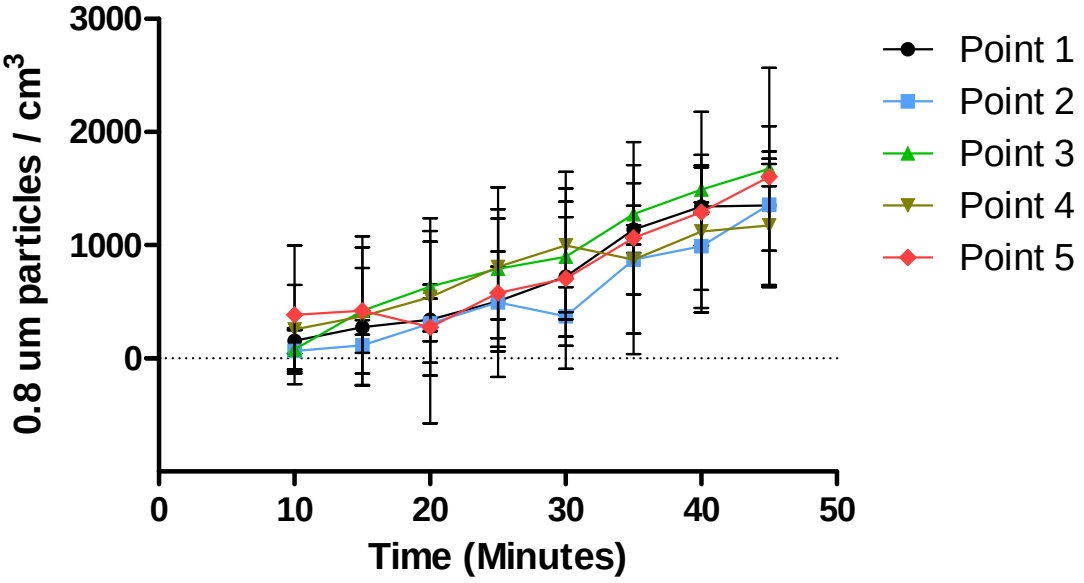

Figure 9. 0.8-µm Bead Concentration of Each Point over Time at a 50-LPM Challenge (*n* = 3)

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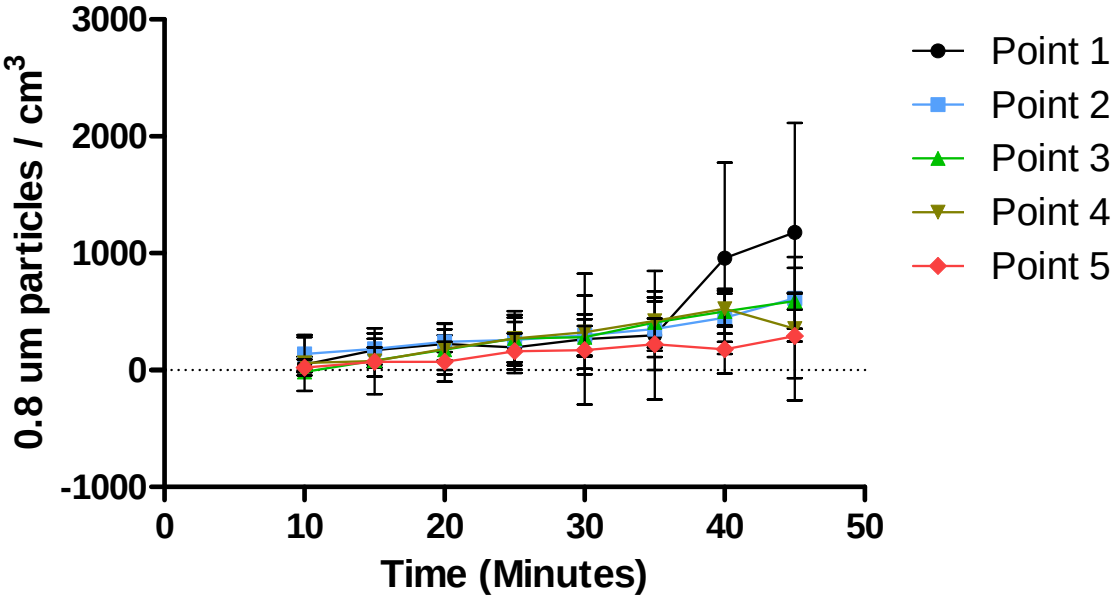

Figure 10. 0.8-µm Bead Concentration of Each Point over Time at an 80-LPM Challenge (*n* = 3)

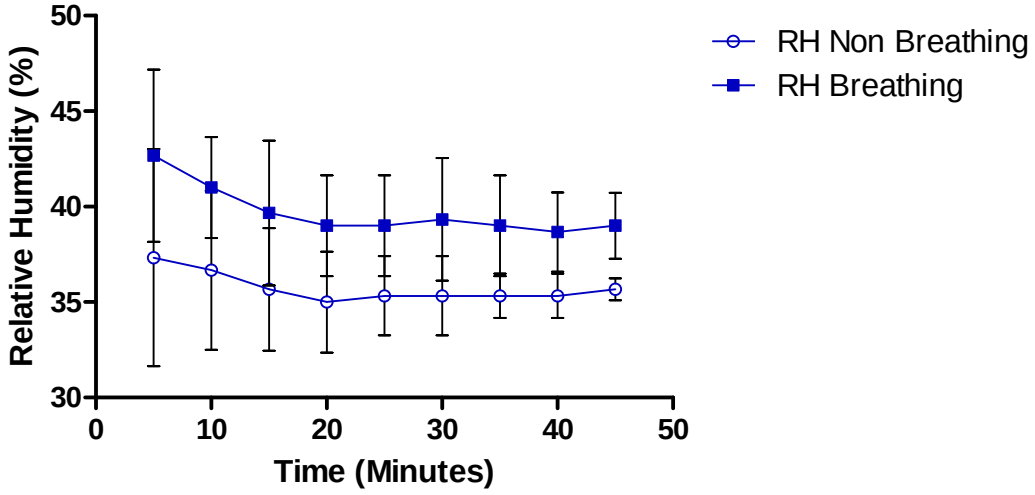

Figure 11. Relative Humidity of a 50-LPM Challenge over Time with and without Breathing (*n* = 3)

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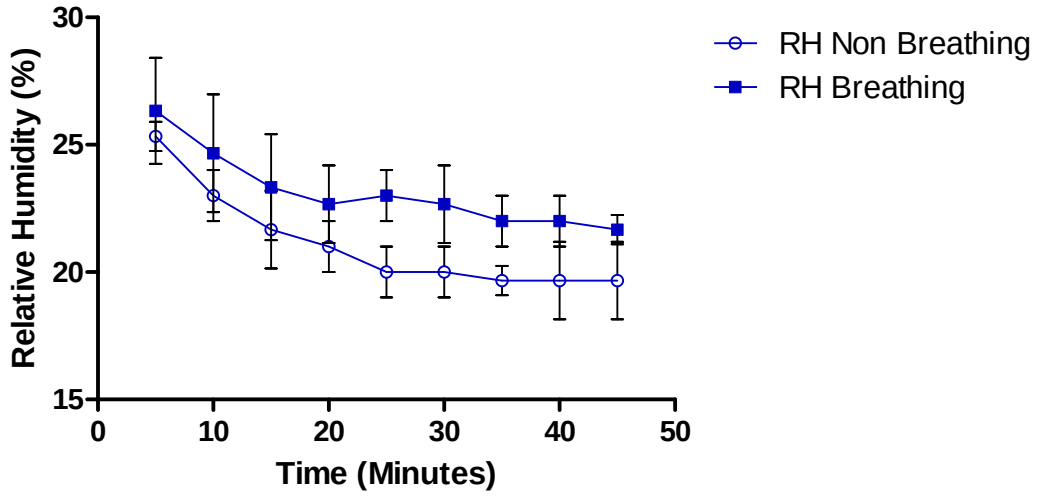

Figure 12. Relative Humidity of an 80-LPM Challenge over Time with and without Breathing (*n* = 3)

The increasing temperatures (Figures 13 and 14) over time of the 50- and 80-LPM challenges during breathing conditions can be attributed to the heat generated from the breathing machine pump and the confined space of the hood.

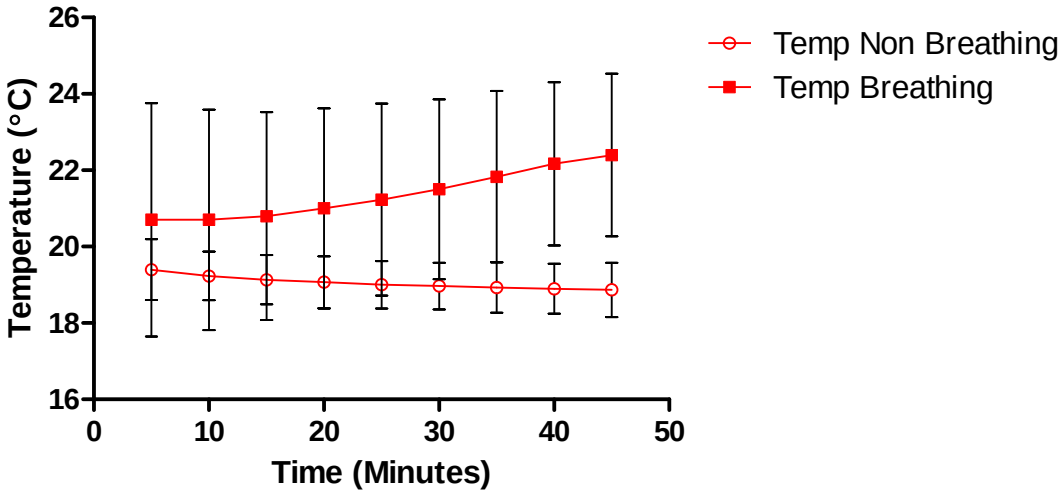

Figure 13. Temperature of a 50-LPM Challenge over Time with and without Breathing (*n* = 3)

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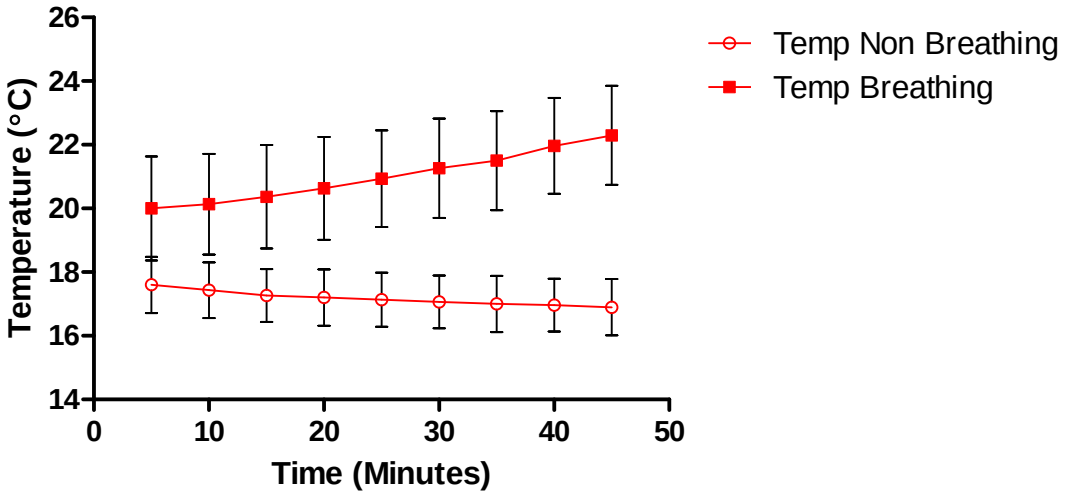

Figure 14. Temperature of an 80-LPM Challenge over Time with and without Breathing (*n* = 3)

The inert bead challenge of the system demonstrated that at both the normal and deep breathing rates, the sampling efficiency of the downstream port compared to the upstream port was 80–85% and remained constant over the 45-min sampling period (Figure 15). While a higher efficiency is more desirable, the data were corrected to account for the difference in sampling efficiency and also corrected for the breathing (downstream port data were divided by 0.83 and multiplied by 2).

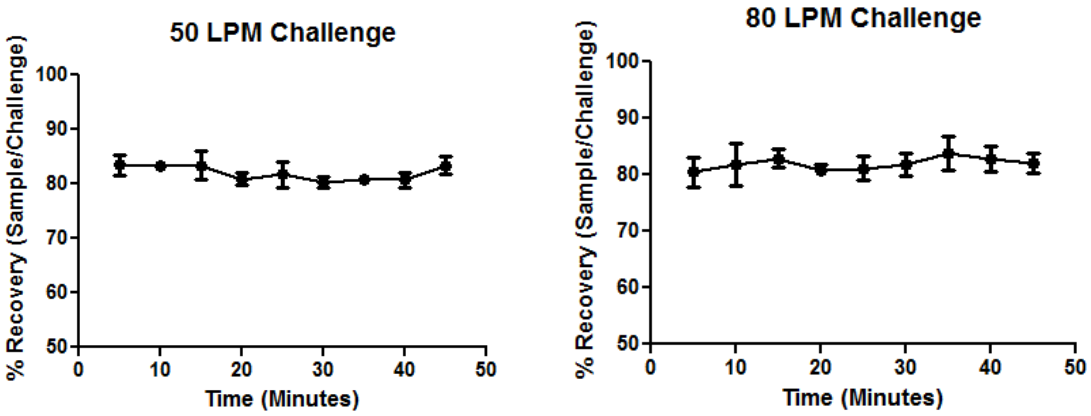

Figure 15. Sampling Efficiency of the Upstream and Downstream Sampling Ports for the Breathing Machine Using a 0.8-µm Bead Challenge.

For each paired test, the H1N1 data demonstrated the difference in sampling between ports was within 0.5 log, which is typically the sensitivity of the $TCID_{50}$ assay (Table 4). The downstream samples were lower in each case, indicating a potential bias in the system. However, the system

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will be capable of discriminating multi-log differences that would be expected in an FFR that does not fit well.

Table 4. H1N1 Influenza Assay Results from Upstream and Downstream Sampling Ports

	Upstream ($TCID_{50}$/L)				Downstream ($TCID_{50}$/L)			
	A	**B**	**C**	**Mean**	**A**	**B**	**C**	**Mean**
Run 1	4.3	5.3	5.3	5.0	4.7	5.2	4.9	4.9
Run 2	5.8	5.3	6.1	5.7	5.4	4.9	5.4	5.2
Run 3	5.8	6.1	6.3	6.1	5.2	5.7	5.9	5.6
Mean				5.6				5.3

*Downstream samples were corrected for breathing (multiplied by 2) and corrected for sampling difference (divided by 0.83).

3.4 Conclusions

The breathing machine was shown to perform adequately to measure total inward leakage of the N95 FFRs with an H1N1 challenge.

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4.0 MS2 CHALLENGE OF REACTIVE MEDIA

4.1 Technical Introduction

The spread of respiratory disease caused by airborne viruses via natural transmission or the intentional dissemination for bioterrorism purposes has led to the development of air filtration media containing antimicrobial technology. Multiple studies have been performed to evaluate the effectiveness of treated filters against biological aerosols, but no studies that we know of have been found to evaluate the antimicrobial effect on the aerosolized microorganism over time while in the aerosol state. The study described herein evaluates the antimicrobial effect of an iodine-treated filter on viable MS2 aerosols when kept in the aerosol state for various lengths of time.

4.2 Methods

4.2.1 Preparation of Virus

MS2 bacteriophage (ATCC® 15597-B1™) was selected as a representative virus aerosol. MS2 is a non-enveloped, icosahedron-shaped, 27-nm, single-stranded RNA virus that infects only male *Escherichia coli* [13]. MS2 was propagated according to standard protocol [14]. Briefly, a swab of a 16-hr culture of *E. coli,* grown at 35 °C and 220 rpm, was added to 100 mL of specialized medium (1% tryptone, 0.5% yeast extract, 1% sodium chloride) along with 0.5 mL of 1 M calcium chloride and incubated for 2.5 hr at 35 °C and 220 rpm, reaching mid-log phase. 1.5 mL of stock MS2 was added to the 2.5-hr subculture and incubated for 16 hr at 35 °C and 220 rpm. After overnight incubation, 5 mg lysozyme was added and incubated for 30 min under the same conditions. 0.2 mL of filter-sterilized 0.5 M ethylenediaminetetraacetic acid (EDTA) and 0.2 mL of chloroform were then added to the culture and incubated another 30 min under the same conditions. The bacterial culture was then centrifuged at 4 °C and 10,200 rpm. Supernatant was removed and filtered through a 0.2-μm pore filter, producing the MS2 suspension (~10^{11} PFU/mL).

4.2.2 Aerosol Test System

A custom aerosol system was designed to deliver aerosolized MS2 through a filter and into a reservoir, keeping the MS2 in the aerosol state (Figure 16). Briefly, a Collison nebulizer (BGI, Waltham, MA) containing 30 mL of stock MS2 diluted 1:10 in 1X phosphate buffered saline (PBS) generated an aerosol that was passed through a Kr-85 charge neutralizer at 11.3 LPM, then through a custom filter holder for 37-mm filter coupons, and finally into a 24-in × 10-in heat-sealed plastic bag for 40 sec for an aerosol sample. Samples were incubated at room temperature, if necessary, then evacuated into an AGI-30 containing 20 mL of sodium thiosulfate. Optional diffusion dryers containing silica gel desiccant beads were added as needed to modify the humidity level obtained.

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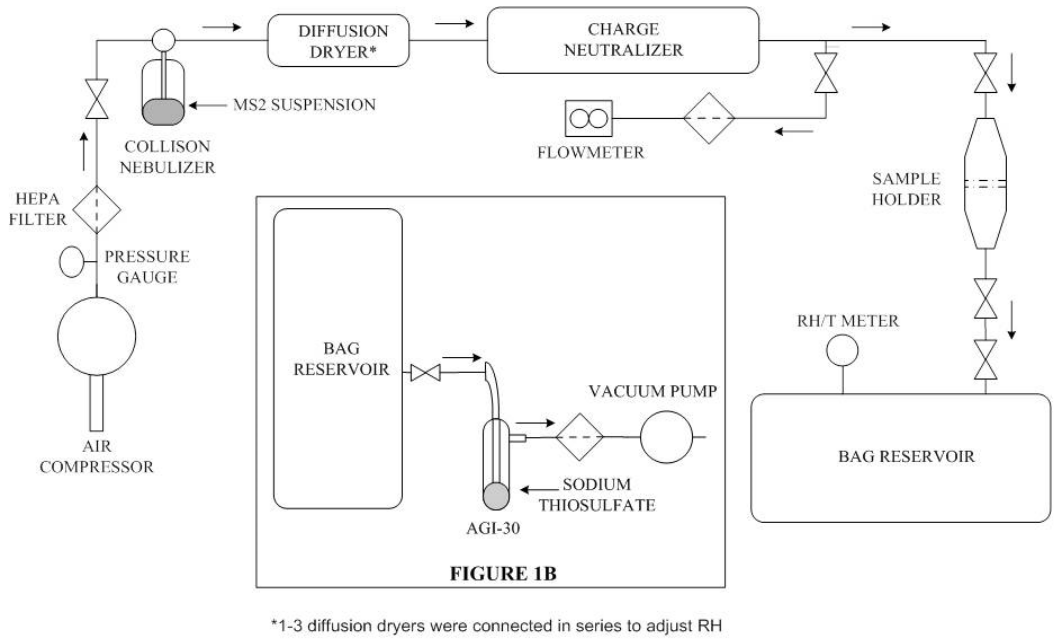

Figure 16. Bag Test System

4.2.3 Test Specifications

Three test conditions were originally proposed to determine MS2 viability in the aerosol state after passing through a treated filter: Condition 1 (high humidity, >80%), Condition 2 (medium humidity, ~50%, and Condition 3 (low humidity, ~25%). Condition 1 has already been reported [2]; Conditions 2 and 3 were investigated as part of this study. Two filter media were tested: an untreated P100 FFR (3M 8293, St. Paul, MN) and an iodine-treated TSH filter.

4.2.4 Virus Enumeration

A plaque assay was performed to enumerate the MS2 according to standard protocol [14]. Briefly, an overnight *E. coli* culture was grown at 35 °C and 220 rpm in 50 mL of tryptic soy broth. The overnight culture was then diluted 1:10 in 1X PBS. 3 mL of the *E. coli* dilution was added to 50 mL of specialized media and incubated for 2.5-hr under the same conditions. Soft agar media was prepared (1% tryptone, 0.1% yeast extract, 0.8% sodium chloride, 0.1% dextrose, 1% agar), kept in a 60 °C water bath, then 0.5 mL of 1% thiamine and 2 mL of 1 M calcium chloride were added per 500 mL of agar medium. Agar medium was mixed then divided as 9-mL aliquots into 15-mL polypropylene tubes and kept in a 55 °C water bath.

MS2 samples were serially diluted 1:10 into sterile 1X PBS tubes. When 2.5-hr incubation ended for the *E. coli* subculture, the plaque assay was performed within 30 min after the 2.5- hr period to maintain optimal *E. coli* conditions. Agar tubes were removed from the water bath in triplicate and briefly allowed to cool without allowing agar solidification. 0.25 mL of *E. coli* was added to each tube then 1 mL of the appropriate dilution was added. Tubes were closed and thrice inverted in gentle fashion. Each tube was then poured into a 100-mm Petri dish, swirled to cover the bottom, and allowed to set for ~15–20 min. All plates were inverted and incubated overnight at 35 °C. Plaques were enumerated using a standard colony counter.

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4.2.5 Data Analysis

Four incubation periods (t = 1, 2, 5, and 10 min) were evaluated for each humidity level. A minimum of three samples were obtained for each incubation period at each humidity level. A 0-min time point sample was taken right before each test sample to provide a reference to which the percent loss in viability could be calculated. For each run, an aerosol sample without a filter was obtained. Each impinger sample was diluted and plated in triplicate. Percent viability remaining was determined as:

$$\% \text{ Viability Remaining} = \frac{P_n}{P_0} \times 100 \quad (6)$$

Where P_n = total plaque forming units for test bag incubated at $t = n$, and P_0 is the 0-min reference sample. A two-tailed t-test was performed comparing the filter types for each humidity condition. A one-way analysis of variance was performed to compare the humidity data for each filter type.

4.3 Results

The mean aerosol concentration used to challenge the filters was $9.25 \pm 6.46 \times 10^9$ PFU/L. For Condition 1, the percent viable remaining of aerosolized MS2 after challenging the P100 and TSH filters ranged from 64–88% and 3–28%, respectively (Figure 17). For Condition 2, the percent viable remaining of aerosolized MS2 after challenging the P100 and TSH filters ranged from 105–107% and 5–20%, respectively. For Condition 3, the percent viable remaining of aerosolized MS2 after challenging the P100 and TSH filters ranged from 99–105% and 28–63%, respectively.

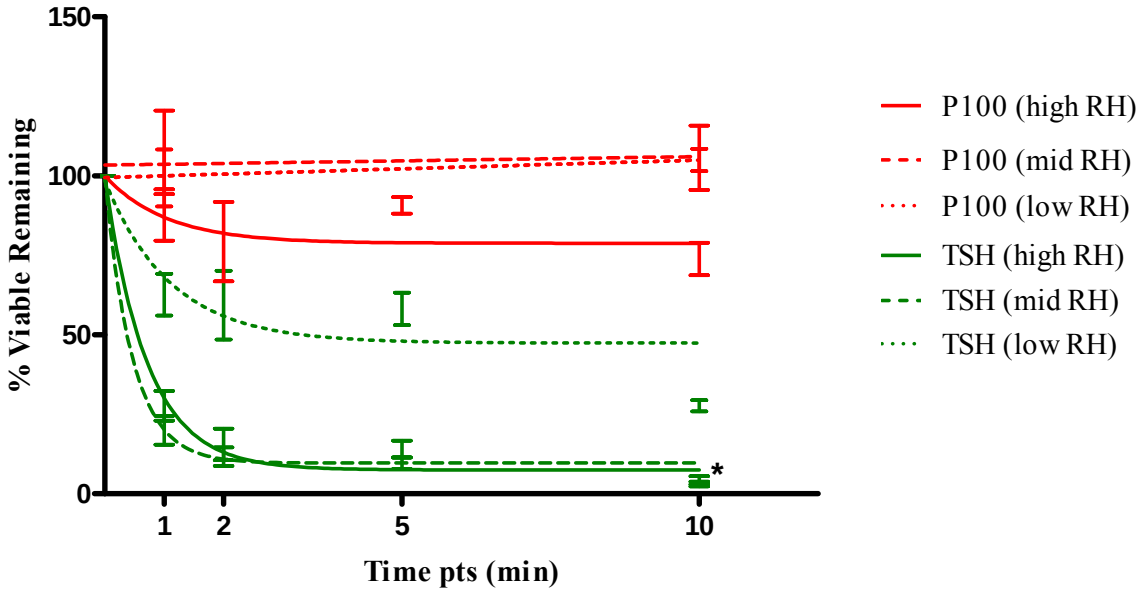

Figure 17. Effect of Humidity on MS2 Viability over Time

*Results from the incubated bag from this time point were below detectable limits

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Statistical analysis indicated significant differences between each filter type for all three humidity levels ($P < 0.001$). Significant differences were found between the three humidity levels for both the P100 ($P = 0.002$) and the TSH filter ($P < 0.001$).

4.4 Discussion

A comparison of the two filter types showed significant differences for all three humidity levels. The higher kill observed for higher humidities was expected as antimicrobial efficacy generally increases in the presence of water. Non-enveloped viruses like MS2 tend to have better environmental stability at higher humidities and vice versa for enveloped viruses, reinforcing the decrease in viability is attributable to the presence of iodine rather than environmental instability. Further studies are needed to identify the mechanism by which the iodine is killing the MS2, whether by physical attachment or vapor concentration.

4.5 Conclusions

This study demonstrates that the iodinated TSH filter has a significant antimicrobial effect on viable MS2 aerosols. The data indicate that as relative humidity increases, the effectiveness of the iodine also increases. Additionally, longer incubation periods result in greater loss of viability when passed through the treated filter.

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5.0 FFR HOSPITAL WEAR STUDY

5.1 Technical Introduction

Hospital-acquired infections affect 5–10% of U.S. hospitalizations. Environmentally stable pathogenic and commensal microorganisms acquire antibiotic resistance and remain infective for long periods of time. Viable pathogens occur in hospital air in concentrations of possible concern, fluctuate with traffic patterns, and require a different risk assessment from those on surfaces. OSHA mandates that hospitals implement a respiratory protection plan for workplace hazards involving respiratory threats. However, respiratory protection standards are typically enforced only when exposure to aerosolized pathogens is a concern from high-risk patients. Volunteers from the environmental staff at Bay Medical Center (Panama City, FL) wore N95 FFRs while cleaning rooms of discharged patients. Contaminating species of bacteria found on the worn FFRs were extracted and characterized based on Gram staining, visual observation, and API analysis [2]. Antibiotic resistance testing was replicated and described herein.

5.2 Methods

147 Gram-positive and 49 Gram-negative isolates were tested for antibiotic resistance according to standard protocols [15].

5.3 Results

Antibiotic resistance was demonstrated by 141 of 196 isolates tested. Oxacillin-resistance was identified in 73% of Gram-positive isolates and 67% of Gram-negatives (Table 5). Vancomycin resistance was found in only 8% of Gram-positives. Resistance to both antibiotics was demonstrated by 7% of tested isolates.

5.4 Discussion

Of the 26 bacterial species recovered from used FFRs, most are coagulase-negative *Staphylococci* (CNS) and *Micrococcus* spp. Of the 196 isolates tested, 72% showed antibiotic resistance to at least one antibiotic. Resistance profiles of CNS have increased, escalating rates of human infections [16]. Resistant CNS may acquire virulence factors by horizontal gene transfer or serve as reservoirs to pass antibiotic-resistant genes to virulent strains. Further study of antibiotic-resistant CNS is needed to characterize their role in hospital acquired infections.

5.5 Conclusions

These data suggest that antibiotic resistant microorganisms are present in hospital air, but the related risk to occupants in hospitals (staff and visitors) is hard to define. More research is needed to evaluate this observation.

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Table 5. Antibiotic Resistance of Isolates Recovered from Contaminated FFRs.

		% Resistant to		
Gram	**Bacterial Species**	**Oxacillin**	**Vancomycin**	**Both**
+	*Kocuria kristinae*	67	0	0
	Kocuria varians/rosea	50	25	+25
	Micrococcus spp.	94	14	14
	Staphylococcus aureus	67	0	0
	Staphylococcus auriculans	100	0	0
	Staphylococcus capitis	50	0	0
	Staphylococcus caprae	100	0	0
	Staphylococcus chromogenes	50	0	0
	Staphylococcus cohnii ssp *cohnii*	50	0	0
	Staphylococcus epidermidis	63	0	0
	Staphylococcus haemolyticus	89	11	11
	Staphylococcus hominis	61	0	0
	Staphylococcus lentus	100	50	50
	Staphylococcus saprophyticus	75	0	0
	Staphylococcus schleiferi	0	0	0
	Staphylococcus sciuri	60	0	0
	Staphylococcus warneri	100	0	0
	Staphylococcus xylosus	100	0	0
	Not Identified	71	24	24
	Total Gram Positive (*n*=147)	**73**	**8**	**7**
–	*Acinetobacter baumannii/calcoaceticus*	100	-	-
	Ochrobactrum anthropi	100	-	-
	Pseudomonas fluorescens/putida	100	-	-
	Rahnella aquatilis	100	-	-
	Stenotrophomonas maltophilia	0	-	-
	Not Identified	63	-	-
	Total Gram Negative (*n*=49)	**67**	**-**	**-**

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LIST OF SYMBOLS, ABBREVIATIONS, AND ACRONYMS

AGI	all-glass impinger
APS	aerodynamic particle sizer
ARA	Applied Research Associates, Inc.
ATCC	American Type Culture Collection
CDC	Centers for Disease Control
CMD	count median diameter
CNS	coagulase-negative *Staphylococci*
DoD	Department of Defense
DHHS	Department of Health and Human Services
EDTA	ethylenediaminetetraacetic acid
EMEM	Eagle's minimum essential medium
FFR	filtering facepiece respirator
GSK	GlaxoSmithKline, Corp.
HEPA	high-efficiency particulate air
LSAT	Laboratory-Scale Aerosol Tunnel
LPM	liters per minute
MDCK	Madin–Darby canine kidney
MS2	MS2 bacteriophage
NIOSH	National Institute for Occupational Safety and Health
OSHA	Occupational Safety and Health Administration
PBS	phosphate buffered saline
PFE	inert particle filtration efficiency
PFU	plaque-forming units
PSD	particle size distribution
PSL	polystyrene latex
RH	relative humidity
RPM	rotations per minute
Sf-EMEM	serum-free Eagle's minimal essential medium
SMPS	scanning mobility particle sizer
$TCID_{50}$	median tissue culture infectious dose assay
TSH	Triosyn Super HEPA
UTC	Universal Technology Corporation
VFE	viable filtration efficiency
WHO	World Health Organization

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